

Supplementary information

Title:

Combined treatment with a pH-sensitive fusogenic peptide and cationic lipids achieves enhanced cytosolic delivery of exosomes

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Figure caption

Supplementary Figure 1. Secretion of CD63-GFP-exosomes from HeLa cells. (a) Confocal microscopic observation of CD63-GFP-HeLa cells or wildtype HeLa cells (without expression of CD63-GFP). Scale bar: 20 μ m. (b–d) Western blot showing exosomes secreted from HeLa cells. The CD63 (b), GFP (c) and CD9 (d) exosome marker proteins were detected as described in the Materials section. Detection of immunoreactive species using anti-CD63 and anti-GFP were the same position at approximately 50 kDa. (e) TEM observation of isolated CD63-GFP-exosomes. Scale bar: 100 nm.

Supplementary Figure 2. Cationic lipids increase the internalisation of exosomes into HeLa cells. (a) Confocal microscopic observation of HeLa cells treated with CD63-GFP-exosomes (20 μ g/ml) in the presence or absence of Lipofectamine LTX (1–4% (v/v)) for 24 h at 37 °C (blue: Hoechst33342, green: CD63-GFP-exosomes). Scale bar: 20 μ m. (b) Colocalization of CD63-GFP-exosomes (20 μ g/ml) in the presence of Lipofectamine LTX (2% (v/v)) and endosome marker DiD was analysed using a confocal microscope after the treatment for 6 h at 37 °C (green: CD63-GFP-exosomes, red: DiD). Arrows show representative colocalization of CD63-GFP-exosomes and DiD. Scale bar: 5 μ m.

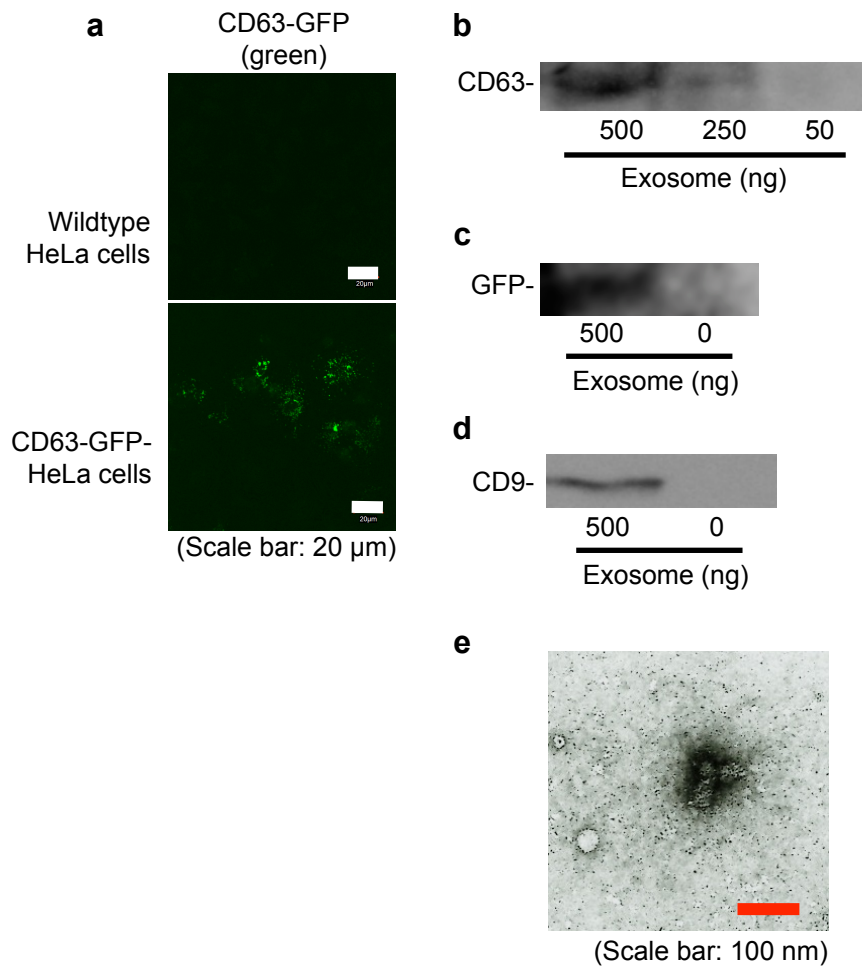
Supplementary Figure 3. Enhanced cellular uptake of exosomes by treatment with cationic lipids. (a) Confocal microscopic observation of CHO-K1 cells treated with CD63-GFP-exosomes (20 μ g/ml) in the presence or absence of Lipofectamine LTX (2 or 4% (v/v)) for 24 h at 37 °C (blue: Hoechst33342, green: CD63-GFP-exosomes). Scale bar: 20 μ m. (b) Relative cellular uptake of CD63-GFP-exosomes (20 μ g/ml) in CHO-K1 cells in the presence or absence of Lipofectamine LTX (2 or 4% (v/v)) analysed using a flow cytometer under the same experimental conditions as (a). (c) Cytotoxicity in the combined treatment of exosomes (20 μ g/ml) and Lipofectamine LTX (2–8% (v/v)) for 24 h at 37 °C on CHO-K1 cells analysed by a WST-1 assay. The data represent the averages (\pm SD) of three (b) and four (c) experiments.

Supplementary Figure 4. Combination treatment with cationic lipids increases cellular uptake of exosomes. (a) Relative cellular uptake of CD63-GFP-exosomes (1–20 μ g/ml) in HeLa cells in the presence or absence of Lipofectamine LTX (0.5–2% (v/v)) for 24 h at 37 °C

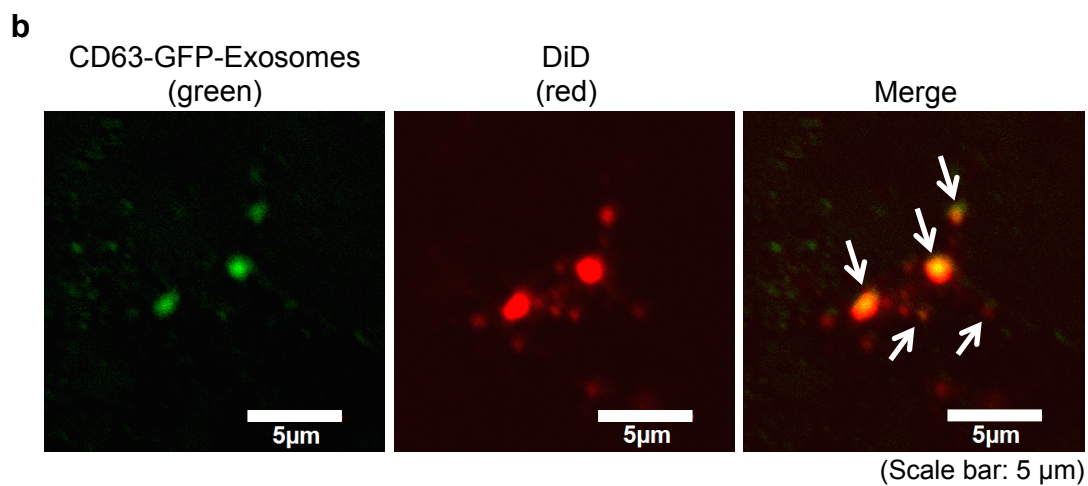
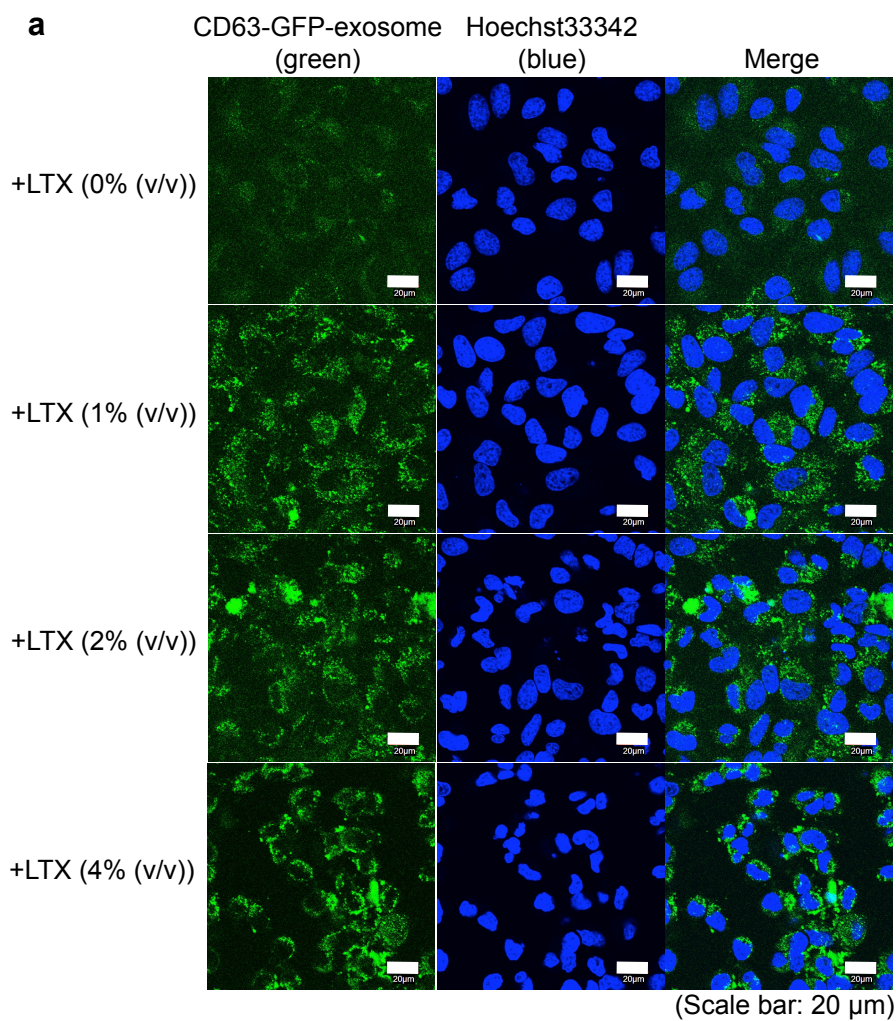
analysed using a flow cytometer. **(b)** Cytotoxicity in the combined treatment of exosomes (1–10 µg/ml) and Lipofectamine LTX (0.5–4% (v/v)) for 24 h at 37 °C on HeLa cells analysed by a WST-1 assay. The data represent the averages (\pm SD) of three **(a)** and four **(b)** experiments.

Supplementary Figure 5. Concentration of GALA peptide affects the cytosolic diffusion efficiency. **(a)** Confocal microscopic observation of HeLa cells treated with exosomes (without expression of CD63-GFP, 5 µg/ml) and FITC-GALA (0.05, 0.5 or 2.5 µM) in the presence of Lipofectamine LTX (0.5% (v/v)) for 6 h at 37 °C (blue: Hoechst33342, green: FITC-GALA). Scale bar: 20 µm. **(b)** Observation of HeLa cells treated with CD63-GFP-exosomes (5 µg/ml) and GALA (without fluorescent label, 0.5 µM) in the presence of Lipofectamine LTX (0.5% (v/v)) for 6 h at 37 °C (green: CD63-GFP-exosomes). Scale bar: 10 µm. **(c)** HeLa cells were treated with exosomes (without expression of CD63-GFP, 5 µg/ml), FITC-GALA (0.5 µM), and Lipofectamine LTX (0.5% (v/v)) in the presence or absence of NH₄Cl (50 mM) for 6 h at 37 °C, prior to confocal microscope observation. Scale bar: 20 µm. **(d)** Confocal microscopic observation of HeLa cells treated in combination with TR-dex encapsulated exosomes (5 µg/ml), FITC-GALA (0.5 µM), and Lipofectamine LTX (0.5% (v/v)) in the presence or absence of NH₄Cl (50 mM) for 6 h at 37 °C (green: FITC-GALA, red: TR-dex). Scale bar: 10 µm.

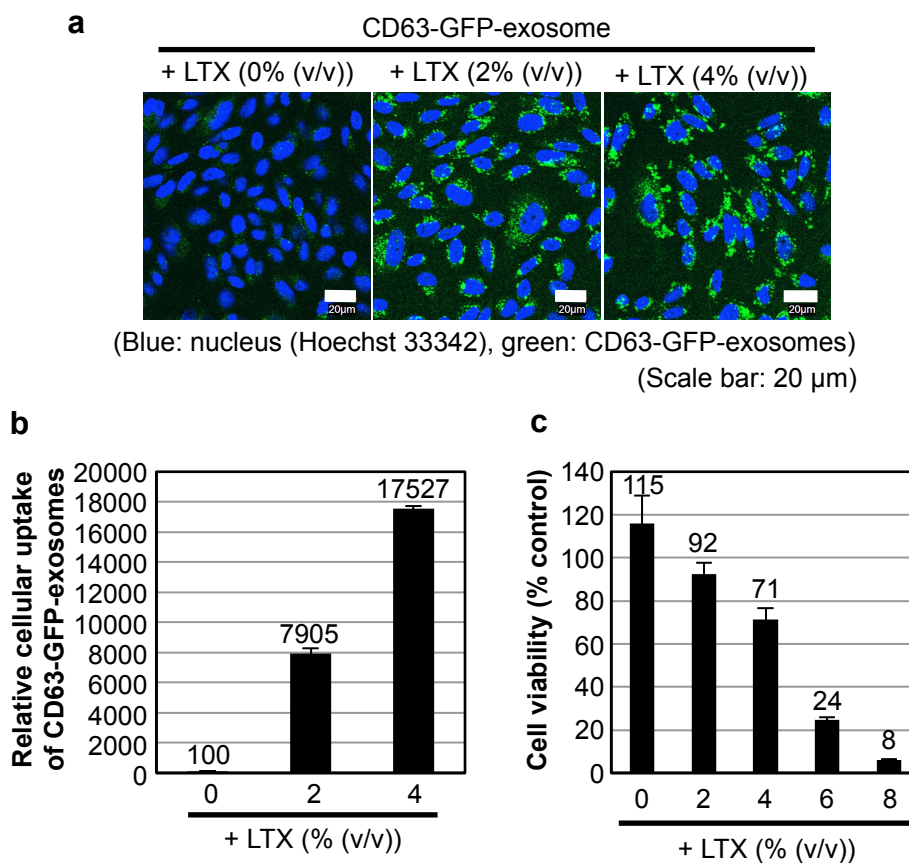
Supplementary Figure 6. Cellular uptake of encapsulated Texas red-dextran in exosomes treated with cationic lipids and GALA peptide. Confocal microscopic observation of CHO-K1 cells treated with a combination of TR-dex encapsulated exosomes (20 µg/ml) and FITC-GALA (0, 2 or 10 µM) in the presence of Lipofectamine LTX (2% (v/v)) for 6 h at 37 °C (green: FITC-GALA, red: TR-dex). Scale bar: 20 µm.



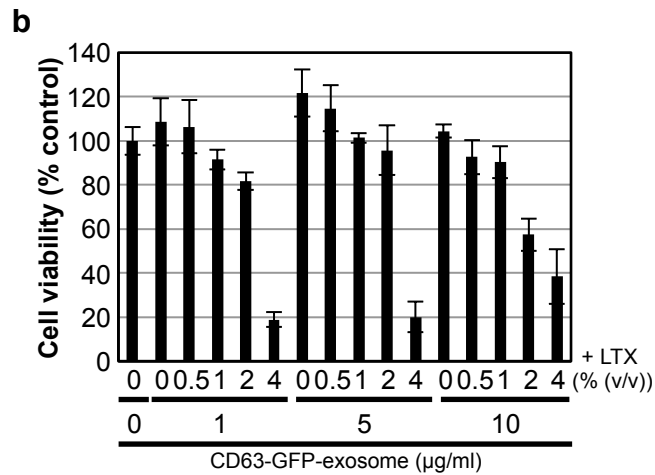
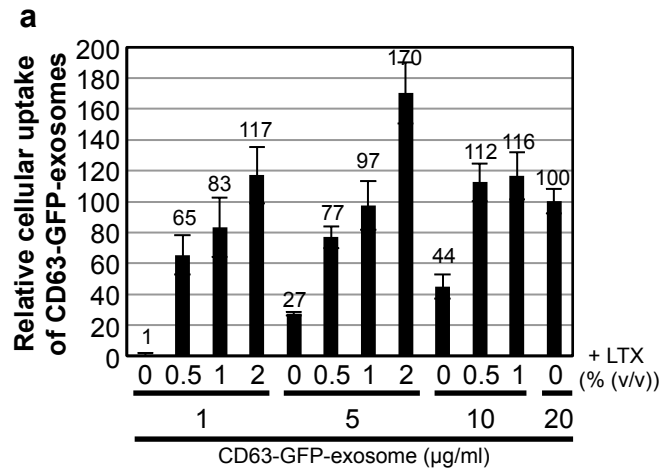
Supplementary Figure 1



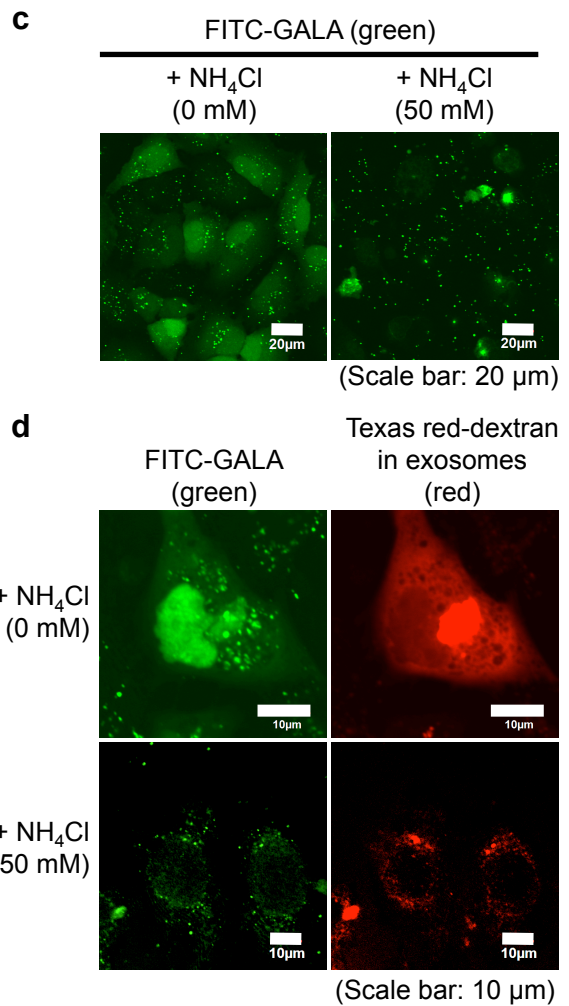
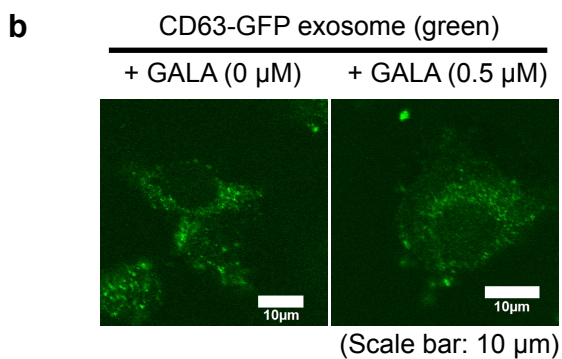
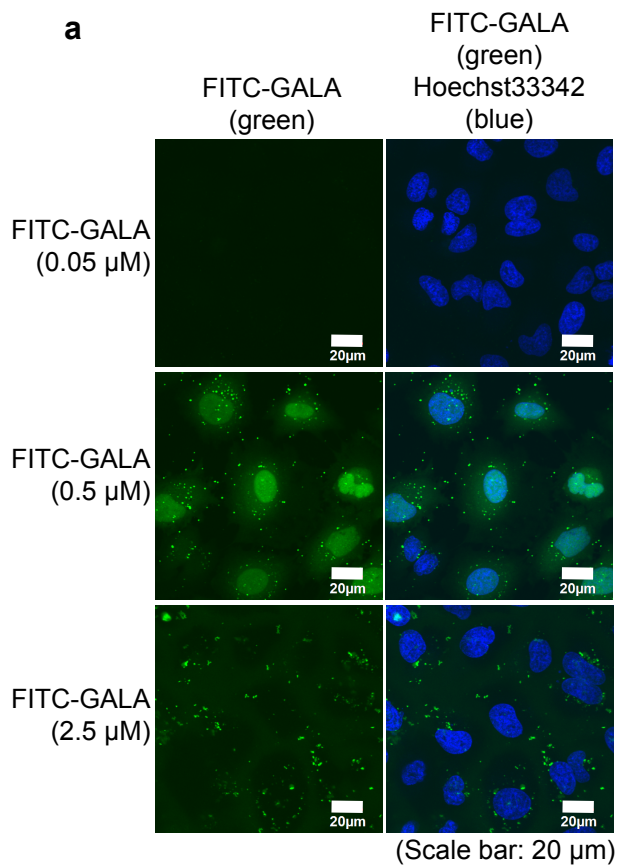
Supplementary Figure 2



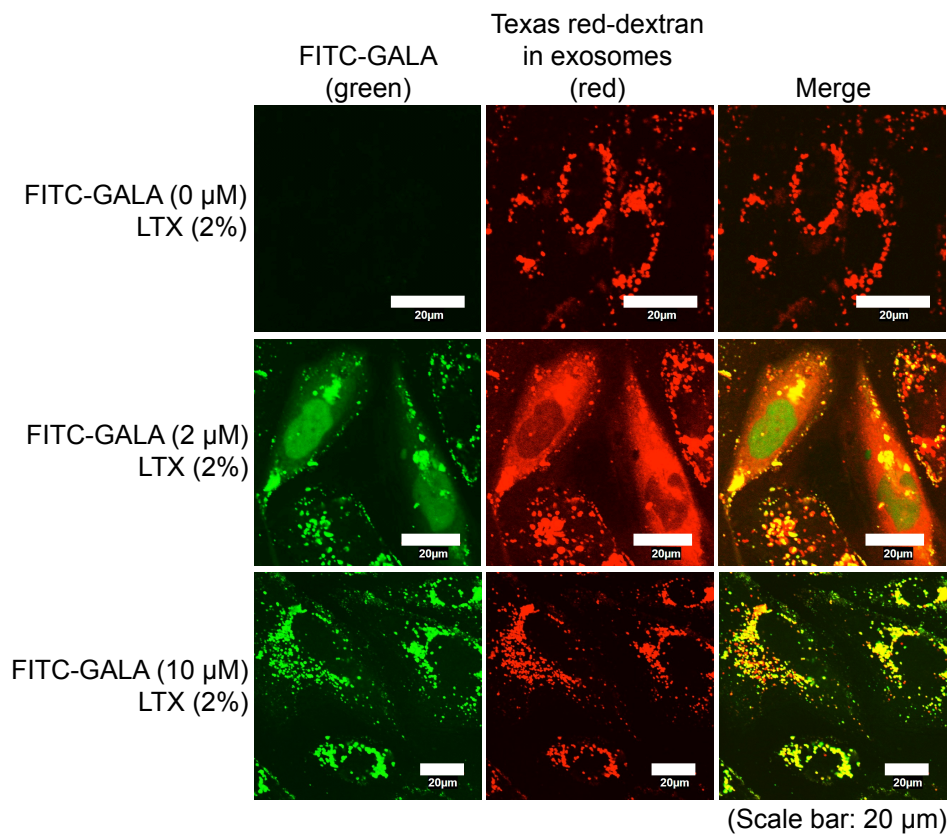
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6